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Abstract: The control of meal size is the best studied aspect of the control of energy balance, and manipulation of this system constitutes a promising target to treat obesity. A major part of this control system is based on gastrointestinal hormones such as glucagon-like peptide-1 (GLP-1) or amylin, which are released in response to a meal and which limit the size of an ongoing meal. Both amylin and GLP-1 have also been shown to increase energy expenditure in experimental rodents, but mechanistically we know much less how this effect may be mediated, which brain sites may be involved, and what the physiological relevance of these findings may be. Most studies indicate that the effect of peripheral amylin is centrally mediated via the area postrema, but other brain areas, such as the ventral tegmental area, may also be involved. GLP-1's effect on eating seems to be mainly mediated by vagal afferents projecting to the caudal hindbrain. Chronic exposure to amylin, GLP-1 or their analogs decrease food intake and body weight gain. Next to the induction of satiation, amylin may also constitute an adiposity signal and in fact interact with the adiposity signal leptin. Amylin analogs are under clinical consideration for their effect to reduce food intake and body weight in humans, and similar to rodents, amylin analogs seem to be particularly active when combined with leptin analogs.

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Gut hormones like amylin and GLP-1 in the control of eating and energy expenditure

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Abstract

The control of meal size is the best studied aspect of the control of energy balance, and manipulation of this system constitutes a promising target to treat obesity. A major part of this control system is based on gastrointestinal hormones like glucagon-like peptide-1 (GLP-1) or amylin which are released in response to a meal and which limit the size of an ongoing meal. Both amylin and GLP-1 have also been shown to increase energy expenditure in experimental rodents but mechanistically, we know much less how this effect may be mediated, which brain sites may be involved, and what the physiological relevance of these findings may be. Most studies indicate that the effect of peripheral amylin is centrally mediated via the area postrema but other brain areas, like the ventral tegmental area may also be involved. GLP-1's effect on eating seems to be mainly mediated by vagal afferents projecting to the caudal hindbrain. Chronic exposure to amylin, GLP-1 or their analogues decrease food intake and body weight gain.

Next to the induction of satiation, amylin may also constitute an adiposity signal and in fact interact with the adiposity signal leptin. Amylin analogs are under clinical consideration for their effect to reduce food intake and body weight in humans, and similar to rodents, amylin analogues seem to be particularly active when combined with leptin analogues.

Keywords: amylin, GLP-1, leptin, satiation, energy expenditure, hormone interaction

Introduction

The pancreatic B-cell hormone amylin and the gut-derived hormone glucagon-like peptide-1 (GLP-1) are released in response to food intake. Behaviorally, both hormones produce similar responses on eating and both hormones have the potential to reduce body weight when administered chronically. In fact, the GLP-1 analogue liraglutide was recently approved as body weight lowering drug by the Federal Drug Administration in the USA.

This review which is based on a presentation given at the Quebec Symposium on Obesity in November 2014, will briefly discuss some recent findings on amylin versus GLP-1 action. An extensive literature search on the topic of this review was carried out by using Pubmed (http://www.ncbi.nlm.nih.gov/pubmed/). As will be discussed, and despite similar behavioral effects after amylin or GLP-1 administration, there seem to be important differences in the mechanisms that lead to the reduction in eating by amylin versus GLP-1.

Production site and secretion of amylin and GLP-1

It is generally believed that pancreatic beta-cells are the major source of circulating amylin and that meal-associated fluctuations of circulating amylin levels directly reflect changes in beta-cell secretion. These fluctuations and the postprandial increase in circulating amylin are the physiological basis for amylin's effect on eating, in particular its effect on meal size [1, 2]. We recently measured levels of amylin and insulin in hepatic portal vein blood samples because this vascular bed best reflects the secretion of beta-cell products into the circulation. The meal induced increase in circulating amylin occurs within a few minutes after meal onset and parallels the increase in plasma insulin[3].

GLP-1 is secreted from enteroendocrine cells that line the entire intestinal epithelium. The density of the GLP-1 producing L-cells increases in more distal parts of the small intestine and in the colon, however the total number of L-cells, at least in rats, is highest in the jejunum, including its proximal part[4]. L-cells express a large number of receptors or transporters that trigger L-cell secretion in response to a variety of stimuli; these include glucose, long or short chain fatty acids but also bile acids that act on the TGR5 receptor[5]. Which of these stimuli contributes most to the postprandial release of GLP-1 is still a matter of debate, in particular in individuals undergoing Roux-en-Y gastric bypass surgery (RYGB) who have largely elevated

82 secretions of postprandial GLP-1[6-11]. GLP-1 is also produced in a subset of neurons
83 in the nucleus of the solitary tract (NTS). The exact role of GLP-1 released from
84 these neurons is still under investigation but they seem to be involved in the
85 mediation of reduced eating in response to aversive stimuli or in sickness anorexia[12].
86 Further, recent data indicate that locally released GLP-1 also contributes to the
87 physiological control of eating and body weight (e.g., [13-15]) because the knockdown of
88 GLP-1 in the NTS leads to increased eating, body weight and adiposity[16].
89

Amylin and GLP-1 as satiation signals

The best investigated function of amylin is the role as a signal of satiation [17]. Amylin is believed to be a physiological controller of meal size [18, 19] because it meets the critical criteria for a physiological endocrine satiation signal. One important criterion is that the meal-contingent infusion of amylin into the portal vein dose-dependently reduced the size and duration of the ongoing meal and that the onset of this action occurred within minutes after administration[2]. The meal size effect of amylin appeared to be independent of the route of administration (e.g., [20-22]), and similar observations have also been reported for GLP-1[23, 24].

Administration of the amylin antagonist AC187 increased meal size[25], underlining the physiological relevance of amylin's effect. While the GLP-1 antagonist exendin-9 has been reported to increase eating under some conditions[26], a specific effect of exendin-9 on meal size has not been observed consistently and may be weak (e.g., [27-29]). Finally, chronic administration of amylin, GLP-1 or their analogues have been shown to reduce body weight by reducing food intake[30-32], and at least in the case of amylin, this was associated with decreased meal sizes over extended time periods[30].

Sites of amylin and GLP-1 action

Amylin and GLP-1 produce similar activation patterns in the caudal hindbrain when assessed by c-Fos immunohistochemistry (e.g., [33-36]) but the primary sites of action may differ between amylin and GLP-1. Most experiments support the idea that the satiating effect of peripheral amylin is mediated by direct humoral action on the area postrema (AP) in the hindbrain which lacks a functional blood brain barrier[25, 30, 37-40]. This evidence is e.g. based on experiments showing that amylin's effect is abolished in rats with AP lesions but not by disrupting afferent nerve signaling from the periphery to the brain[41-44]. Further, AP administered amylin antagonists blocked the anorectic action of peripheral amylin[25].

Recent experiments indicate that the AP may not be the only primary receptive site for the action of peripheral amylin or its analogues, and that the ventral tegmental area (VTA) may also play a role in this respect[45]. The peripheral administration of the amylin receptor agonist salmon calcitonin (sCT) reduces eating by activating amylin receptors[46], and this effect is blocked by the VTA administration of the amylin antagonist AC187[47]. How amylin (or sCT) may reach VTA neurons is unclear; the

VTA is protected by the blood brain barrier but amylin transport across the blood brain barrier has been described[48, 49] so that direct VTA activation by peripheral amylin or sCT seems possible. It is however important to note that the rat amylin-1 receptor is activated equally by amylin and the neurotransmitter calcitonin gene-related peptide (CGRP)[50], and, importantly, that the effects of both peptides at the amylin-1 receptor are blocked by AC187. Hence, it cannot be excluded that primary activation of AP neurons may trigger CGRP release in the VTA to explain the observations discussed above.

In contrast to amylin, the acute effect of GLP-1 to reduce eating may be due to a paracrine effect on intestinal vagal afferents which transmit the signal to the nucleus of the solitary tract (NTS) which is adjacent to the AP. This finding is mainly based on the observation that the effect of intraperitoneal (but not intravenous) GLP-1 was blocked by subdiaphragmatic deafferentation, a technique which blocks all vagal afferent signaling from the abdomen to the brain[23]. Whether a direct action of GLP-1 on the AP[51] also plays a role under physiological conditions is still a matter of debate. Interestingly, amylin and GLP-1 sensitive AP neurons seem to constitute different populations of neurons because amylin receptors are found in amylin activated but not in GLP-1 activated AP cells[34]; hence the AP may be able to discriminate between effects of different signals even though their behavioral effect on eating is similar.

Amylin and GLP-1 receptor function

The amylin receptor is composed of a heterodimer of the calcitonin receptor (CTR) core protein that combines with one or several receptor activity modifying proteins (RAMPs) to yield specific amylin receptors[52-54]. Receptor binding and mapping studies have shown a wide distribution of the amylin receptor components throughout the central nervous system, and a high density of both the CTR and RAMPs is found in the AP[55-58]. Recent experiments in our laboratory have shown that single amylin activated AP neurons contain all necessary components of the functional amylin receptor 1 or 3, i.e. CTR plus RAMP1 or CTR plus RAMP3, respectively; in fact, AP neurons may often contain both types of RAMPs within single cells[59]. The functional difference of amylin sensitive AP neurons containing the amylin1, the amylin 3, or the amylin 1/3 receptor is currently unknown.

The presence of fully functional amylin receptors in the AP is consistent with the co-expression of cyclic GMP (cGMP) which is one of the second messengers of amylin receptor activation[25, 60], in CTR carrying AP neurons[34]. Another second messenger system activated by amylin is the ERK/MAPK system. Amylin leads to a phosphorylation of ERK, and this effect may be involved in the rapid effects of amylin on eating because at least under certain conditions, inhibition of ERK phosphorylation prevented the effect of amylin[61].

Part of the amylin activated AP neurons seem to express dopamine-beta-hydroxylase (DBH) which characterizes noradrenergic neurons. In fact, roughly 50% of amylin activation seems to occur in neurons expressing DBH[39, 62] while the phenotype of the remainder of amylin activated neurons is unclear; at least part of them may be second order neurons which therefore do not necessarily express amylin receptors and the amylin signaling transduction machinery themselves.

Interestingly, even though circulating GLP-1 also may directly activate AP neurons[51] and even though the general brain activation pattern after amylin or GLP-1 injection shows many similarities and a large overlap among affected regions[33], amylin sensitive AP neurons seem to form a population of neurons that is different from GLP-1 sensitive AP neurons; this is based on the presence or absence of the CTR in amylin versus GLP-1 activated AP neurons, respectively [34]. Further, GLP-1's eating inhibitory action seems to differ between fasted versus fed animals because GLP-1 decreased eating when administered to rats after refeeding with a 3g meal, but not when administered in the fasted state[63]; amylin, in contrast, has been shown to reduce eating when administered to fasted or ad libitum fed animals (e.g., [43, 62, 64]). The increased effectiveness of GLP-1 to reduce eating in refed animals may be related to an increase in the GLP-1 receptor translocation to the cellular membrane of vagal afferent neurons; the cell bodies of these neurons are located in the nodose ganglion. These neurons mediate the satiating effect of GLP-1[23] but the increased effectiveness of GLP-1 in refed animals (which coincides with this receptor translocation) may indicate that GLP-1 also controls postprandial satiety[63].

Effects of amylin and GLP-1 on energy expenditure

Energy balance is determined by gross energy intake, energy expenditure and energy loss via faeces, fermentation gases or urine. Here, I briefly want to summarize effects of amylin and GLP-1 on energy expenditure, all reported results are based on the assessment of energy expenditure by indirect calorimetry. Generally, manipulations that result in changes of eating or body weight are often accompanied by alterations in energy expenditure. Usually, body weight reduction by dieting leads to an adaptive physiological response to reduce energy expenditure; this response helps the body to minimize the potential negative effects of long term energy restriction in a state of negative energy balance ("starvation response"). Interestingly, both amylin and GLP-1 may at least partly counteract this response.

Some of the earlier studies showed that acute administration of the amylin receptor agonist sCT increased energy expenditure in the absence of food[65, 66]; further, chronic peripheral administration of amylin also increased energy expenditure in rats and this effect was paralleled by a decrease in eating and body weight gain. Further, the effect of amylin to increase energy expenditure was markedly enhanced in mice

overexpressing the amylin receptor component RAMP1 which indicates an important role of the amylin-1 receptor; this effect seemed to be paralleled by increased activation of the sympathetic outflow to enhance brown adipose tissue thermogenesis[67]. The latter effect is in line with experiments showing that the effect of peripheral or central amylin on energy expenditure can be blocked by co-administration of a beta-adrenergic receptor antagonist[68].

The site of amylin action for these effects has not yet been investigated in detail but the AP may play some role; acute injections of amylin or sCT into the AP increased energy expenditure at a dose approximately 1000 times lower than peripherally effective doses. During chronic administration, amylin infused into the AP was also able to prevent the decrease in energy expenditure seen in rats whose food intake was yoked to the amylin treated rats[69]. The brain pathways linking the presumed primary site of action in the AP[69] and enhanced sympathetic output[67] are currently unknown.

Similar to amylin, GLP-1 or its agonists also seem to increase energy expenditure under some but not all[70] experimental conditions; the effect is dose dependent and seems to be most robust when GLP-1 is administered centrally[71]. Interestingly, a recent study also indicated that GLP-1 may be involved in the control of energy expenditure in humans because the inhibition of GLP1-breakdown by a dipeptidyl peptidase IV inhibitor increased energy expenditure in men[72].

Overall, these studies indicate that amylin and GLP-1 seem to modulate energy metabolism both via an effect on food intake and on energy expenditure, but the former effect is characterized far more extensively than the latter.

Interactions of amylin with other factors involved in the control of energy metabolism

Amylin and leptin

Consistent with the concept that long term “adiposity signals” may modulate the effectiveness of meal associated satiation (“short term”) signals[73, 74], a number of recent studies investigated the interactions between amylin and leptin. One of the first studies in respect to amylin reported that rodent models with a defective leptin signalling system have a reduced response to anorectic doses of the amylin agonist sCT[75]. Subsequently, we reported that acute administration of leptin to the third ventricle increased the eating-inhibitory effect of peripheral amylin [76].

Interest in this type of interaction was fueled by the finding that amylin may be able to reduce the leptin resistance that is commonly associated with obesity [77-80]. Leptin resistant obese rats were "re-sensitized" to leptin by chronic amylin administration [81]. In other words, amylin which itself is still effective in obese rats[3, 82, 83] reduced eating and body weight significantly more when leptin was co-administered with amylin[81, 84-86]. The effect of amylin on leptin's action and leptin sensitivity appeared to be specific to amylin [81] because the effects were not seen to the same extent with the GLP-1 analog AC3174 [81], or when infusions of leptin were combined with the GLP-1 receptor agonist exendin-4 [87]. Leptin combined with GLP-1 analogs did produce stronger effects on eating and body weight than single compounds, but the interaction seemed to be (mathematically) additive rather than synergistic [88]. Further, and in contrast to the amylin-induced sensitization of animals to leptin, the effect was only present in animals that had already lost a substantial amount of weight or after animals were switched from an obesogenic high fat diet to regular rodent chow [89]; hence, manipulations which themselves may affect leptin sensitivity.

The amylin/leptin combination also had increased effects on energy expenditure [81]. The effect of the amylin/leptin combination on energy balance appeared to be paralleled by a preferential oxidation of fat as indicated by the low respiratory quotient in both the amylin/leptin and the pair-fed groups [81, 84-86]; importantly, the lower respiratory quotient was still evident in amylin/leptin co-injected animals during the weight stable phase and not only during weight loss like in the pair-fed group [81, 85, 90-92].

All effects combined, the amylin/leptin combination treatment prevented the suppression of energy metabolism that is typically seen in situations of negative energy balance which may e.g. be induced by simple dieting. The potential mechanisms of this interaction have been summarized recently[85, 93, 94]. Briefly, most data indicate that the hypothalamus, and in particular the ventromedial hypothalamus is critically involved in this interaction. Amylin strongly enhanced leptin signalling specifically in the ventromedial hypothalamus, and this was also confirmed under in vitro conditions [81, 85 95 96, 97]. Amylin also increased leptin binding in the ventromedial hypothalamus and other hypothalamic sites, e.g. the dorsomedial hypothalamus (DMH) [85] while leptin receptor expression was reduced in the mediobasal hypothalamus in amylin-deficient mice [85].

A recent study indicated that interleukin-6 (IL-6) seems to be involved in the leptin-sensitizing effect of amylin. Amylin induced the increased synthesis and release of IL-6 from hypothalamic microglia which seems to act on leptin-receptor positive neurons in the ventromedial hypothalamus to improve hypothalamic leptin signaling. This was corroborated by the finding that rats treated with antibodies against IL-6 or mice deficient for IL-6 did not show the same enhancing effect of amylin on leptin signaling compared to their respective controls [98].

Finally, and consistent with earlier studies that leptin-deficient ob/ob mice are less sensitive to sCT [75], we recently showed that leptin receptor deficient db/db mice or Zucker ZDF rats respond less to acute amylin injections than respective wildtype controls; further, the reduction of body weight and adiposity by leptin was lower in amylin-deficient mice than in wildtype controls [85], and amylin-deficient mice had less leptin induced pSTAT3 formation in the ventromedial hypothalamus [85]. In other words, endogenous leptin action may be required for a full action of amylin and the presence of amylin signaling may mutually be necessary for the full effect of leptin.

Amylin and CCK

Next to the interaction between amylin and leptin, the combined effects of amylin and CCK on eating has attracted most interest. Amylin and CCK reduce eating mainly by a meal size effect and their combined administration leads to a stronger reduction in eating than single administration[99, 100]. The effect seems to be synergistic because ineffective doses of amylin and CCK combined to produce near maximal reductions in eating.

A series of experiments indicated that CCK's anorectic action may be partly mediated by amylin and that amylin is a necessary modulator of CCK's effect because the eating inhibitory effect of CCK can be attenuated by amylin receptor antagonists [101, 102]. Further, amylin was necessary for the full eating inhibitory effect of CCK because CCK's action was nearly abolished in amylin deficient compared to control mice; this effect could be rescued because co-administration of a subthreshold dose of amylin with CCK restored normal CCK responsiveness[103].

Amylin and estradiol

Food intake in mammals is sexually differentiated and estradiol plays the major role in gender specific effects in females. One important effect of estradiol is to increase the effectiveness of satiating hormones like CCK; this effect, e.g., contributes to the cyclic decrease in eating in female rats on their day of estrus[104-106].

Trevaskis and colleagues [107] were the first to test the effect of amylin in female rats specifically in the presence or absence of estradiol. Surprisingly, eating and body weight in ovariectomized rats was reduced more by chronic amylin than in intact control rats or in ovariectomized rats receiving physiological estradiol replacement. Body adiposity also tended to be reduced by amylin in the ovariectomized compared to sham operated or estradiol replaced rats [107]. The mechanisms underlying the effect of estradiol remained unclear but it was shown that ovariectomized rats had reduced neurogenesis in particular in the AP and that chronic amylin restored this effect. Theoretically, increased neurogenesis may lead to an increase in the number of amylin receptors or amylin responsive cells in the AP, but this remains to be studied.

More recent data from our own lab indicate that the interaction between amylin and estradiol seems more complex. Under conditions of acute amylin administration, single amylin injections reduced eating *more effectively* in estradiol replaced rats than in ovariectomized rats without physiological estradiol replacement[108, 109]. Hence, future experiments need to clarify whether the role of estradiol in modulating amylin action depends on the experimental conditions or whether a common mechanism under acute or chronic conditions can be identified.

GLP-1 and estradiol

Similar to CCK and amylin, the eating inhibitory effect of GLP-1 also seems to be enhanced by estradiol because physiological estradiol replacement in ovariectomized rats enhanced GLP-1's action[74]. Further, a recent study showed that non-physiological replacement of estrogen in the form of a GLP-1/estrogen conjugate lead to a stronger decrease in eating and body weight than GLP-1 alone [110]. The mechanisms underlying these effects have not been studied yet.

Amylin as a potential treatment strategy against obesity

Basic research findings on the interaction between amylin and leptin have been described above. Because obese animals and humans are often leptin resistant and hence unresponsive to exogenous leptin, the finding that amylin increases leptin sensitivity and that amylin may therefore be able to overcome leptin resistance in obese individuals is of high clinical relevance. Clinical trials tested the combined use of the amylin analogue pramlintide as adjunct therapy with insulin for the treatment of type 1 and type 2 diabetes; these trials showed that treatment of diabetic persons with insulin plus pramlintide improved glycemic control and also lead to a significant body weight loss compared to insulin monotherapy [111]. Pramlintide was subsequently shown to reduce energy intake in type 2 diabetics and obese non-diabetics[112-115]. Similar to experiments in rodents, the combination of the amylin and leptin analogues pramlintide and metreleptin, respectively, was effective in lowering body weight and adiposity in humans [81, 116]. The clinical data were encouraging and future work will have to test the effects of prolonged treatment, potential side effects, and the consequences of cessation of treatment. Similar to treatment of diabetics with insulin, the maintenance of body weight loss may require continuous therapy because the weight lowering effect seems to fade on discontinuation of treatment (see also [92, 93, 117]).

Pramlintide releasing fat sensors

Recently, an interesting experimental approach to reduce eating and body weight has been reported[118]. The authors of this study produced a self-controlled release device for the amylin analogue pramlintide. Cells were manipulated in a way that they contained a closed-loop genetic circuit which constantly monitored blood fatty acid levels and which was coupled to the coordinated and reversible expression and release of pramlintide. The fatty acid sensor was based on the peroxisome proliferator-activated receptor-α. This sensor which was sensitive to a broad spectrum of fatty acids, was subsequently shown to be activated in a reversible manner in vitro and also in vivo, e.g. when manipulated cells were administered to mice as intraperitoneal implants. Most importantly, increasing amounts of dietary fat led to an enhanced release of pramlintide which resulted in reduced eating and body weight in mice on a high fat but not on a low fat diet[118]. Whether this strategy can be employed clinically needs to be studied in coming years.

Summary

This review briefly summarizes some recent findings in respect to the control of eating and body weight by amylin and GLP-1. Both hormones or their respective analogues also seem to be active in humans. While the use of amylin analogues, in particular in combination with leptin, is still at the experimental phase, the GLP-1 analogue liraglutide has recently been approved for anti-obesity treatment in the USA.

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